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# Useful Primer Designs to Amplify DNA Fragments of the Plastid Gene *mat*K from Angiosperm Plants

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Although the plastid gene *mat*K can be a useful tool of molecular systematics using nucleotide sequences, there were few primer designs for the polymerase chain reaction with broad applicability. Here we report the new primer designs to amplify and sequence the *mat*K fragment from various groups of angiosperms.

Plant molecular systematics using nucleotide sequences of the *rbc*L (e.g. Chase et al. 1993) has got a great success in elucidating phylogenetic relationship of plants at various levels. However, the *rbc*L gene is conservative and the phylogenetic tree reconstructed by the *rbc*L data has often little resolution especially when closely related species were compared.

The plastid $\text{tRNA}^{\text{Lys}}$ gene *trn*K has a 2.5 kb class II intron incuding a 1.5 kb open reading frame called *mat*K. It was suggested to code a maturase for splicing the precursor of the *trn*K (Neuhaus and Link 1987). Several recent studies suggested that nucleotide sequence of this gene is useful to resolve the phylogenetic relationship within angiosperm families. Using the *mat*K, Johnson and Soltis (1994) successfully analyzed the phylogenetic relationship in the Saxifragaceae sensu stricto. Steele and Vilgalys (1994) analyzed Polemoniaceae. These two studies showed the primer sequences to amplify the *mat*K gene from their materials. However, using their primers we could not amplify *mat*K fragments from some other angiosperms. Thus, we tried to design new primers with broad applicability.

Our comparative study for the *rbc*L and *mat*K sequences suggests that the *rbc*L gene has hot spots that accelerate amino acid replacement while most of non-synonymous sites are conservative (Ooi et al. in preparation). Frequent nucleotide changes in particular sites decrease the reliability of the phylogenetic reconstruction under the maximum parsimony method. On the other hand, such hot spots are not found in the *mat*K. Moreover the mutation rate of the *mat*K is two to three times higher than the *rbc*L. Thus, the *mat*K gene is more useful for the phylogenetic reconstruction within angiosperm families than the *rbc*L. Here we present new primer designs and their range of applicable species to amplify *mat*K fragments.

## Materials and Methods

We searched conservative regions by comparing the *mat*K sequence of tobacco (Sugita et al. 1985) with that of rice (Hiratsuka et al. 1989). The tobacco sequence in the regions were chosen for the primers. In the course of the primer design, we took care that the sequence length and GC content was not so different each other, that no palindrome existed in the sequence, and that the 3' terminal sequences avoided to compose the primer dimmers.

To amplify the DNA fragments, we used the following conditions. Total genomic DNA was extracted by the CTAB method (Doyle and Doyle 1987). For PCR reaction, we used a programmable temperature control system PC-700 (Astec, Japan) and following cycle profiles: 1 min at 94°C for once, 1 min at 94°C, 1 min at 45°C, 2 min at 72°C for 30 cycles, and 15 min at 72°C. The reaction mixture was prepared using *TaKaRa Taq* DNA polymerase and its instruction (TaKaRa Co. Ltd., Japan).

## Results and Discussion

Table 1 shows the primer designs. The *mat*K-8R was modified from the complementary sequence of the *mat*K8 (Steele and Vilgalys 1994), and the others were newly designed in this work. Any combination from two forward primers and two reverse primers are possible to provide the *mat*K fragments. For example, the *mat*K-AF and *mat*K-8R provide a 1.2 kb fragment and the *mat*K-BF and *mat*K-R provide a 0.9 kb fragment (Fig. 1).

Figure 2 shows the range of applicable species. The species were selected from different clades (monocots, Asteridae and Rosidae) of the phylogenetic tree inferred from the *rbc*L sequence data (Chase et al. 1993). We could amplify *mat*K only from *Chrysosplenium* and *Ficus* in the 8 species listed in the Fig. 2 using the combination of *mat*K-1412F and *mat*K-1848R or *mat*K-2200R (Johnson and Soltis 1994). On the other hand, our attempts to amplify *mat*K from both monocots and dicots were mostly successful, although we could not amplify *mat*K from gymnosperms and ferns.

The PCR products will be purified and applied to

Table 1. Location and base composition of the primers. Location indicates the start and end nucleotide positions relative to the tobacco sequence (Sugita et al. 1985)

| Primer | 5' sequence 3' | Location |
|---|---|---|
| *mat*K-AF | CTA TAT CCA CTT ATC TTT CAG GAG T | 804-828 |
| *mat*K-BF | TCA GAG GGA TTT GCG TTT ATT GTG G | 1038-1062 |
| *mat*K-R | CTG CAT ATA CGC CCA AAT CGG TCA A | 1990-1966 |
| *mat*K-8R | AAA GTT CTA GCA CAA GAA AGT CGA | 2080-2057 |

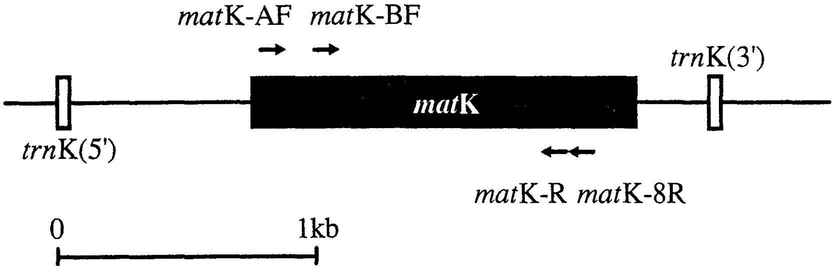

Fig. 1. Relative position of the primers designed in this study. Arrows indicate the direction of strand synthesis. A darkened box represents the coding region.

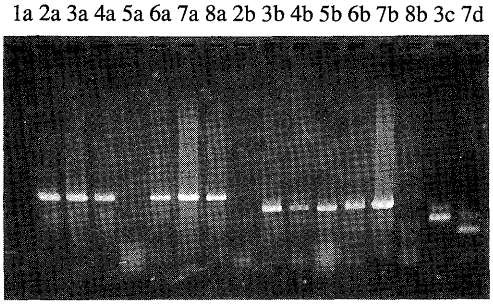

Fig. 2. Photograph of agarose-electrophoresed PCR products from various species using the primers designed in this study. The number and the letter of each lane indicate species and the primer combination as follows; 1: *Gnetum parvifolium* (Gnetaceae), 2: *Oryza rufipogon* (Poaceae), 3: *Lysichiton camtschatcense* (Araceae), 4: *Chrysosplenium flagelliferum* (Saxifragaceae), 5: *Acer pictum* (*A. mono*; Aceraceae), 6: *Ficus nipponica* (Moraceae), 7: *Rehmannia glutinosa* (Scrophulariaceae), 8: *Aster rugulosus* (Asteraceae), a: *mat*K-AF and *mat*K-8R, b: *mat*K-BF and *mat*K-R, c: *mat*K-AF and *mat*K-1470R, d: *mat*K-1412F and *mat*K-8R. 1a, 1b, 2b, 5a and 8b were failed to amplify. 4c, 6c, 7c, 8c and 4d were successfully amplified (not shown).

the direct sequencing using PCR reaction for sequencing with automatic DNA sequencer (Autocycle sequencing kit and A. L. F. DNA sequencer; Pharmacia, Sweden). However, a 0.9 kb fragment is too long to determine the whole sequence by the direct sequencing method. Internal primers are required. Johnson and Soltis (1994) reported that the *trn*K-3914F, *trn*K-2R, *mat*K-1412F and *mat*K-1470R had broad applicability among their primers. We could amplify successfully the *mat*K from both monocots and dicots using the combination of *mat*K-AF and *mat*K-1470R, and from both Rosidae and Asteridae using the *mat*K-1412F and *mat*K-8R. They are good candidates for the internal primer (see Fig. 2). The coding region of the *mat*K can be sequenced directly by the primers designed in this study. The primers enable us to get *mat*K sequence data for phylogenetic reconstruction of particular groups within angiosperms. The primers have been used successfully for phylogenetic reconstruction in several groups of angiosperms (Endo, in preparation; Denda, Komatsu, personal communications). As Johnson and Soltis (1994) remarked in the end of their paper, we also hope that the result of this study stimulate the use of *mat*K sequence data for plant molecular systematics.

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大井和之，遠藤康弘，横山　潤，村上哲明：**被子植物の葉緑体 *mat*K 遺伝子の増幅に有効なプライマー配列**

葉緑体ゲノム上の遺伝子 *mat*K は，*rbc*L に比べて進化速度が速く，塩基配列による種間や属間の分子系統解析に有効であると考えられる．しかしながら現在まで，広い範囲で PCR 法による DNA 断片の増幅ができるプライマー配列はほとんど報告されていない．双子葉類，単子葉類の双方で目的の DNA 断片が増幅できるプライマーを設計したのでここに報告する．